JPPT | Retrospective Observational Study

Performance of Verigene Rapid Diagnostic Testing for Detection of Inpatient Pediatric Bacteremia

Amy Kruger Howard, PharmD; Kimberly Claeys, PharmD; Jessica M. Biggs, PharmD; Kristine A. Parbuoni, PharmD; Kristie Johnson, PhD, D(ABMM); Paige Luneburg, MD; James Campbell, MD, MS; and Jill A. Morgan, PharmD

OBJECTIVE Verigene blood culture panels comprise rapid diagnostic testing, which aids in early bacteremia species identification. This study determined the concordance of Verigene rapid diagnostic results compared with the Vitek reference standard in patients admitted to a children's hospital.

METHODS This was a 3-year retrospective observational study of neonatal and pediatric patients ≤18 years admitted to a children's hospital with confirmed bacteremia for whom Verigene testing was performed. Verigene testing was conducted on cultures with reported growth on Gram stain and final organism speciation confirmed via Vitek. Percent concordance and positive percent agreement with 95% CIs were calculated for Verigene panel-identifiable organisms. Negative percent agreement with 95% CIs was calculated for non-panel organisms. Time-to-result was calculated from Gram stain reporting to both Verigene and Vitek final organism susceptibility.

RESULTS One hundred thirty-five Gram-positive (GP) and 51 Gram-negative (GN) isolates were identified through Vitek. Verigene GP panel-detectable organisms were correctly identified 96.9% (125/129) at the genus level and 95.3% (123/129) at the species level. Overall positive percent agreement was 95.3 (CI: 90.2–98.3). Negative percent agreement was 83.3 (CI: 35.9–99.6) for the 6 non-panel GP organisms. All GN isolates were correctly identified on Verigene. Median time-to-result was 2.9 hours (IQR 2.6, 3.2) and 44.4 hours (IQR: 35.4, 52.5) for Verigene and final susceptibilities, respectively. There was a statistically significant time savings of 41.5 hours (CI: 29.8–53.2) for identification and detection of resistance markers ($p < 0.0001$).

CONCLUSION Verigene concordance at our institution aligns with results from previously published studies and can be considered a reliable clinical decision-support tool.

ABBREVIATIONS BC, blood cultures; CoNS, Coagulase-negative *Staphylococcus*; GN, Gram-negative; GP, Gram-positive; NICU, Neonatal Intensive Care Unit; NPA, negative percent agreement; PICU, pediatric intensive care unit; PPA, positive percent agreement; RDT, rapid diagnostic testing; UMCH, University of Maryland Children's Hospital

KEYWORDS blood culture panels; blood stream infection; Gram-negative organism; Gram-positive organism; pediatrics; rapid diagnostic test; Verigene

J Pediatr Pharmacol Ther 2021;26(5):472–477

DOI: 10.5863/1551-6776-26.5.472

Introduction

Verigene rapid diagnostic tests (RDT) are nucleic acid microarray panels that aid in early identification of Gram-positive (GP) and Gram-negative (GN) organisms, as well as select resistance markers, found in positive blood cultures (BCs). According to Verigene BC-GP and BC-GN package inserts, test results are reported within 2.5 hours of Gram stain detection, and the species-level positive agreement for adult cultures ranges from 93% to 100% and from 92% to 99%, respectively.[1,2] Subsequent studies[3,4] generally support these findings; however, *Streptococcus pneumoniae* concordance has been reported to be as low as 40%.

Only 5 studies (4 BC-GP and 1 BC-GN)[5-9] have been published to date on the ability of Verigene to correctly identify microbial species in pediatric blood cultures; 2 of the 4 BC-GP studies[7,8] were conducted at the same institution with overlapping data collection dates.[5-9] The single BC-GN study[6] reported an 89% overall concordance. Similar to adult data, overall testing concordance for BC-GP ranged from 93% to 96%, with particular species, such as *S pneumoniae*, demonstrating less consistent testing concordance. In one study,[8] only 8 of 33 *S pneumoniae* isolates were correctly identified on the Verigene panel. The reliability of these results is critical if the panels are being used as the basis for clinical therapy modification. At our institution, there was acknowledged hesitancy from clinicians to act on Verigene results.

For this reason, the purpose of this study was to determine the percent concordance of all pediatric Verigene RDT results as compared with the reference standard Vitek results among University of Maryland Children's Hospital (UMCH) patients. Secondary outcomes included calculation of organism-specific concordance, positive percent agreement (PPA) for panel-identifiable organisms, negative percent agreement (NPA) for panel negative organisms, as well as a comparison of time savings for time-to-result for Verigene RDT and final susceptibility reporting. This study adds to the body of literature regarding Verigene RDT testing concordance with traditional microbial identification and reinforces the reliability of the RDT to guide antimicrobial stewardship in clinical decision making.

Materials and Methods

Study Design. This was a retrospective observational study of neonatal and pediatric patients age ≤18 years admitted to the UMCH NICU, PICU, or Pediatric Progressive Care Unit with confirmed bacteremia. The UMCH is housed within the larger University of Maryland Medical Center. At the time of the study, UMCH had no formal antimicrobial stewardship in place to respond to RDT result reporting. All cultures included in the analysis were collected between September 1, 2015, and October 31, 2018. Each sample corresponds to the initial culture drawn for a unique patient with suspected bacteremia during a given hospitalization. Any patient missing culture data based on retrospective chart review or admitted to the University of Maryland Medical Center Adult Trauma service were excluded. Patients with a polymicrobial infection detected on Gram stain were also excluded from the statistical analysis because this is a known limitation of Verigene RDT.[1,2,10] However, full reporting of organisms detected in polymicrobial cultures was included for completeness.

Clinical Specimen Collection and Reporting. Routine blood culture testing at our institution consists of collection in BacTAlert FA and/or FN bottles and initial organism identification through the BacTAlert 3D automated system (bioMérieux, Durham, NC). If the Gram stain identifies in the presence of bacteria, the corresponding Verigene BC-GP or BC-GN (Luminex Corporation, Austin, TX) is run on one blood culture sample from the available set(s). Vitek MS and Vitek 2 (bioMérieux) automated susceptibility testing is performed for organism identification and phenotypic susceptibilities, and results are typically available within 24 to 48 hours of initial Gram stain. The UMCH uses a weight-based protocol for blood culture collection. Most pediatric blood culture samples were ≤4 mL.

Gram stain and Verigene result reporting were documented in the patient's electronic medical record, including the time and date at which the microbiology tech called the patient's provider. Of note, the microbiology lab does not regularly report Verigene resistance mechanisms for Coagulase-negative *Staphylococcus* (CoNS), which is regularly considered to be a culture contaminant, and Vitek speciation and sensitivities are run only at the request of the provider.

The BC-GP panel identifies the following *Staphylococcus* species: *S aureus; S epidermidis; S lugdunensis; coagulase-negative Staphylococcus* (not lugdunensis); *S pneumoniae; S pyogenes; S agalactiae; S anginosus* group; *Streptococcus* spp. other than *S pneumoniae, S anginosus* group, *S pyogenes*, and *S agalactiae*. It also identifies *Enterococcus faecalis, Enterococcus faecium*, and the resistance determinants mecA, vanA, and vanB.[1] The BC-GN panel identifies *Acinetobacter* spp.; *Citrobacter* spp.; *Enterobacter* spp.; *Proteus* spp.; *Escherichia coli; Klebsiella pneumoniae; Klebsiella oxytoca; Pseudomonas aeruginosa*; and resistance determinants KPC, NDM, CTX-M, VIM, IMP, and OXA.[2] Verigene reports BC-GP or BC-GN negative for cultures growing any organism outside of those able to be identified on their respective panels, as detailed above.

Statistical Analysis. Descriptive statistics were reported as mean or median, as appropriate, for patient demographics. Overall percent concordance was calculated for correctly identified BC-GP and BC-GN, including resistance determinants. The PPA for each panel-identifiable organism and NPA for panel negative (Verigene non-detectable) organism along with 95% CIs were calculated using Epitools online calculations and the Clopper-Pearson method of binomial expansion. Time-to-result was calculated from Gram stain to Verigene and Gram stain to Vitek final organism susceptibility for all panel-detectable specimens. A Wilcoxon signed-rank test was calculated for mean time savings using the Vassar Stats computational Web site.

Results

A total of 283 blood cultures were screened, and 226 met study inclusion criteria. After excluding patients admitted to non-pediatric services (n = 25), those for whom we were missing final speciation (n = 14), or those with polymicrobial Gram stain (n = 6), 181 positive blood cultures were analyzed. Patients included had a median age of 1.2 years (IQR: 0.14, 5.65).

A total of 135 GP and 51 GN isolates were identified on Vitek final result—127 GP and 49 GN isolates in 176 monomicrobial cultures and 8 GP and 2 GN isolates in 5 polymicrobial cultures. Of the 135 total GP isolates, 129 were Verigene panel-detectable organisms. For the panel-detectable BC-GP organisms, 96.9% (125/129) of sample isolates were correctly identified at the genus level, and 95.3% (123/129) were correctly identified at the species level. For the panel non-detectable organisms, Verigene correctly reported BC-GP negative in 83.3% (5/6) of isolates: *Micrococcus luteus* (3), *Moraxella* (1), and *Rothia* spp. (1). *Enterococcus avium* was misidentified as *E faecium*. The BC-GP PPA was 95.3 (CI: 90.2–98.3), and the NPA was 83.3 (CI: 35.9–99.6).

Table 1. Performance of Verigene BC-GP RDT for Positive Blood Cultures Compared With Traditional Organism Identification

Organism Identified on Vitek Final Report	BC-GP Correctly Identified	True Positive	False Positive	True Negative	False Negative	Positive Percent Agreement (95% CI)	Negative Percent Agreement (95% CI)
Enterococcus							
E faecium (VRE)	1/1	1	0	0	0	100 (2.5–100)	—
E faecalis	9/9	9	0	0	0	100 (66.4–100)	—
Staphylococcus							
CoNS, not speciated	52/54	52	0	0	2*	96.3 (87.3–99.5)	—
S epidermidis (MSSE)	4/4	4	0	0	0	100 (39.8–100)	—
S hominis	4/4	4	0	0	0	100 (39.4–100)	—
S lugdunensis	1/1	1	0	0	0	100 (2.5–100)	—
S aureus	28/28	28	0	0	0	100 (87.7–100)	—
MRSA	7/7	7	0	0	0	100 (59–100)	—
MSSA	20/21	20	0	0	1†	95.2 (76.2–99.9)	—
Streptococcus							
Alpha-hemolytic *Streptococci*‡	1/2	1	0	0	1§	50 (1.3–98.7)	—
S agalactiae (Group B)	3/3	3	0	0	0	100 (29.2–100)	—
S anginosus group	1/1	1	0	0	0	100 (2.5–100)	—
S gallolyticus‡	2/2	2	0	0	0	100 (15.8–100)	—
S mitis/oralis‡	3/4	3	0	0	1¶	75 (19.4–99.4)	—
S pneumoniae	8/9	8	0	0	1#	88.9 (51.8–99.7)	—
S viridans group‡	6/7	6	0	0	1*	85.7 (42.1–99.6)	—
Other (BC-GP non-panel detectable)	5/6	0	1**	5	0	—	83.3 (35.9–99.6)

BC-GP, blood cultures Gram positive; CoNS, coagulase-negative Staphylococcus; MRSA, methicillin-resistant Staphylococcus aureus; MSSA, methicillin-sensitive Staphylococcus aureus; MSSE, methicillin-sensitive Staphylococcus epidermidis; RDT, rapid diagnostic testing; VRE, vancomycin-resistant Enterococcus

* Misidentified as panel negative on BC-GP.

† Misidentified as MRSA on BC-GP.

‡ BC-GP results denoted as *Streptococcus* spp.

§ Misidentified as CoNS on BC-GP.

¶ Misidentified as *S pneumoniae* on BC-GP.

Misidentified as *Streptococcus* spp. on BC-GP.

** Misidentified as *E faecium* on BC-GP.

Resistance mechanism detection was reported for 42 BC-GP samples with a 97.6% (41/42) concordance. Verigene incorrectly detected mecA resistance for a methicillin-sensitive *S aureus* (1). Table 1 details the number of individual isolates correctly identified and misidentified on the BC-GP panel.

All 37 BC-GN Verigene panel-detectable organisms were correctly identified, including one CTX-M-resistant *K pneumoniae*, for an overall PPA of 100 (CI: 90.5–100; Table 2). All 14 BC-GN negative results were also appropriately negative as confirmed on Vitek final report, with an NPA of 100 (CI: 76.8–100). On Vitek final report Verigene panel negative organisms were as follows: *Morganella morganii* (2), *Achromobacter xylosoxidans* (3), *Ralstonia pickettii* (1), *Bordetella holmesii* (1), *Haemophilus influenzae* (1), *Serratia marcescens* (1), *Bacteroides fragilis* group (1), *Burkholderia* species (1), non-fermenting Gram-negative rod, not *Pseudomonas aeruginosa* (1); *Pseudomonas putida* (1), and *Salmonella paratyphi* A (1).

There were 6 polymicrobial cultures identified on Gram stain. Five cultures had at least 2 organisms identified by Vitek. One culture identified as polymicrobial on Gram stain had only a single species identified by Vitek. Among these 6 polymicrobial cultures, Verigene failed to identify 6 of 12 (50%) organisms (Table 3). However, when including the 5 polymicrobial Vitek cultures with single Gram stains, Verigene misidentified only 6 of 22 (27%) organisms. Assessing the potential clinical impact of changes to treatment secondary to Verigene results revealed that 2 patients would have been insufficiently treated, 1 would have received unnecessary dual coverage, and 3 would not have been affected.

Median time-to-result was 2.9 hours (IQR: 2.6, 3.2) and 44.4 hours (IQR: 35.4, 52.5) for Verigene and final susceptibilities, respectively. For panel-detectable organisms included in the study, there was a statistically significant time savings of 41.5 hours (CI: 29.8–53.2) for identification and detection of resistance markers ($p < 0.0001$).

Table 2. Performance of Verigene BC-GN RDT For Positive Blood Cultures Compared With Traditional Organism Identification

Organism Identified on Vitek Final Report	BC-GN Correctly Identified	True Positive	False Positive	True Negative	False Negative	Positive Percent Agreement (95% CI)	Negative Percent Agreement (95% CI)
Citrobacter freundii	1/1	1	0	0	0	100 (2.5–100)	—
Enterobacter aerogenes	1/1	1	0	0	0	100 (2.5–100)	—
Enterobacter cloacae complex	8/8	8	0	0	0	100 (63.1–100)	—
Escherichia coli	16/16	16	0	0	0	100 (79.4–100)	—
Klebsiella oxytoca	3/3	3	0	0	0	100 (29.2–100)	—
*Klebsiella pneumoniae**	6/6	6	0	0	0	100 (54.1–100)	—
Pseudomonas aeruginosa	2/2	2	0	0	0	100 (15.8–100)	—
Other (BC-GN non-panel detectable)	14/14	0	0	14	0	—	100 (76.8–100)

BC-GN, blood cultures Gram negative

* Includes a single isolate identified as CTX-M resistance.

Discussion

Overall concordance for the BC-GP panel aligns with that reported in prior publications[8,9] with 94% to 96% agreement. The high rate of misidentification among *Streptococcus* species, as noted with *S pneumoniae* in the analysis by Vareechon et al,[8] appears to be an outlier. Other data,[5,9] including our own, suggest that successful detection ranges from 89% to 100% of *S pneumoniae* isolates. Additionally, our 12 of 15 (80%) *Streptococcus* spp. isolates correctly identified align with data published in these same studies. The 3 *Streptococcus* spp. misidentified in our cultures would not have resulted in therapeutic de-escalation; thus, all patients would have been sufficiently covered by empiric therapy until the receipt of the Vitek results.

Our study found a higher concordance for BC-GN samples, at 100%, compared with the 89% previously published rate of identification.[6] The prior BC-GN study, with 104 samples from 2 institutions, included 57 seeded cultures. Verigene RDT does not perform amplification; as such, reliability of panel identification is contingent on the concentration of bacterial growth present in the sample. Unlike seeded samples that guarantee a consistent threshold of bacterial growth, results from clinical samples are more generalizable to real-world practice.

The incidence of polymicrobial cultures was similar to that reported in prior pediatric studies.[5,9] Previous investigations[1,2,10] have cited the limitations of Verigene in polymicrobial detection, and the manufacturer discourages use in these instances. Considering all polymicrobial cultures, 7 of 11 were fully concordant with Vitek, similar to the 68% reported by Beckman et al.[9]

At the time of this study, our institution withheld mecA resistance reporting for CoNS infections because of the high likelihood of CoNS being a contaminant. Despite being less frequently a contaminant in neonates, non-reporting of mecA was unlikely to have affected treatment decisions. Our institutional antibiogram suggests that fewer than 5% of all true CoNS bacteremia are susceptible to oxacillin or cefazolin.

The overall high accuracy of Verigene in terms of resistance mechanism detection should provide clinicians with confidence to tailor therapy earlier, which may result in a clinically significant reduction in morbidity and mortality, antimicrobial resistance, and health care costs.

Conclusion

Verigene RDT at UMCH bolsters the results published previously and supports the notion that Verigene can be considered a reliable clinical decision-support tool for pediatric patients, with the potential to dramatically expedite administration of appropriate antibiotic therapy.

Article Information

Affiliations. Department of Pharmacy Practice and Science, University of Maryland School of Pharmacy, Baltimore, MD (AKH, KC, KAP, JAM); Clinical Pharmacy Practice, University of Maryland Medical Center, Baltimore, MD (KC, KAP, JMB); Pediatrics, University of Maryland School of Medicine, Baltimore, MD (PL, JC); Pathology, University of Maryland School of Medicine, Baltimore, MD (KJ).

Correspondence. Amy Kruger Howard, PharmD; akhoward@rx.umaryland.edu

Table 3. All Polymicrobial Cultures

	Gram-Stain Detection	Verigene Identification	Vitek Identification	Notes on Clinical Impact of Misidentification
1*	GNR	*Acinetobacter* species	*Acinetobacter* species	N/A
	GPC PR + CH	CoNS *Enterococcus faecalis*	CoNS *Enterococcus faecalis*	
2*	GPC PR, CH, + CL	CoNS	Alpha-hemolytic *Streptococcus*, not *pneumoniae*†	Continuation of empiric treatment for CoNS would have sufficiently covered the organisms
	GPC PR, CH, + CL	—	Alpha-hemolytic *Streptococcus*, not *pneumoniae*†	
3*	GNR	*Klebsiella oxytoca*	*Klebsiella oxytoca*	Potential for unnecessary dual coverage until Vitek results available
	GPC PR + CH	*Enterococcus faecalis*	*Klebsiella oxytoca*	
4*	GPC CL	MSSA	MSSA	De-escalated to nafcillin; may have not appropriately covered secondary infection
	GPC PR + CH	—	*Streptococcus viridans* group†	
5*	GPC PR + CH	*Streptococcus pneumoniae*	Alpha-hemolytic *Streptococcus*, not *pneumoniae*	Potential for insufficient coverage until Vitek results available
	GPC PR + CH		CoNS	
6*	GPR	BC-GP negative	*Corynebacterium species*	N/A
	GPC CL	—	—	
7	GPC PR + CH	MSSA *Streptococcus agalactiae* (Group B)	MSSA *Streptococcus agalactiae* (Group B)	N/A
8	GPC CL	CoNS	*Staphylococcus hominis* *Staphylococcus epidermidis*	N/A
9	GPC PR	CoNS *Enterococcus faecalis*	CoNS *Enterococcus faecalis*	N/A
10	GPC PR + CL	CoNS CoNS	*Staphylococcus haemolyticus* *Staphylococcus hominis*	N/A
11	GNR	BC-GN negative	*Achromobacter xylosoxidans* Non-fermenting GRN, not *Pseudomonas aeruginosa*	N/A

BC-GN, blood cultures Gram-negative; BC-GP, blood cultures Gram-positive; CH, chains; CL, clusters; CoNS, Coagulase-negative Staphylococcus; GNR, Gram-negative rods; GPC, Gram-positive cocci; GRN, Gram-negative rod; MSSA, methicillin-sensitive Staphylococcus aureus; N/A, not applicable

* Cultures excluded from Verigene concordance analysis.

† Vitek final sensitivities reported intermediate resistance to penicillin.

Disclosure. Drs. Johnson and Claeys have received research support from BioFire Diagnostics and GenMark Diagnostics and are on the speaker's bureau for Luminex Corporation and GenMark Diagnostics. The other authors declare no conflicts or financial interest in any product or service mentioned in the manuscript, including grants, equipment, medications, employment, gifts, and honoraria. The authors had full access to all patient information in this report and take responsibility for the integrity and accuracy of the report.

Ethical Approval and Informed Consent. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guidelines on human experimentation and have been approved by the University of Maryland Baltimore Institutional Review Board. Given the nature of this study, the project was exempt from the informed consent requirement.

Acknowledgments. Thank you to Caitlin Soto and Caitlin Sas, Student Pharmacists, who assisted in the data collection

process, as well as Hyunuk Seung, MS, for his work on the statistical analysis. Preliminary results were presented at PPA Annual Meeting Resident Project Presentations in Tulsa, OK, on April 11, 2019.

Submitted. July 21, 2020

Accepted. October 29, 2020

References

1. Verigene Gram-positive blood culture nucleic acid test (BC-GP) [fact sheet]. Austin, TX: Luminex Corporation; 2016. Accessed January 10, 2019. http://www.nanosphere.us/sites/default/files/support-docs/nanosphere_bcgp_insert_final.pdf
2. Verigene Gram-negative blood culture nucleic acid test (BC-GN) [fact sheet]. Austin, TX: Luminex Corporation; 2016. Accessed January 10, 2019 http://www.nanosphere.us/sites/default/files/support-docs/nanosphere_bcgn_insert_final.pdf
3. Bork JT, Leekha S, Heil EL, et al. Rapid testing using the verigene Gram-negative blood culture nucleic acid test in combination with antimicrobial stewardship intervention against Gram-negative bacteremia. *Antimicrob Agents Chemother*. 2015;59(3):1588–1595.
4. Wojewoda CM, Sercia L, Navas M, et al. Evaluation of the Verigene Gram-positive blood culture nucleic acid test for rapid detection of bacteria and resistance determinants. *J Clin Microbiol*. 2013;51(7):2072–2076.
5. Sullivan V, Turner NN, Roundtree SS, et al. Rapid detection of Gram-positive organisms by use of the Verigene Gram-Positive Blood Culture Nucleic Acid Test and the BacT/Alert Pediatric FAN System in a multicenter pediatric evaluation. *J Clin Microbiol*. 2013;51(11):3579–3584.
6. Sullivan KV, Deburger B, Roundtree SS, et al. Pediatric multicenter evaluation of the Verigene Gram-negative blood culture test for rapid detection of inpatient bacteremia involving Gram-negative organisms, extended-spectrum beta-lactamases, and carbapenemases. *J Clin Microbiol*. 2014;52(7):2416–2421.
7. Mestas J, Polanco CM, Felsenstein S, Bard JD. Performance of the Verigene Gram-positive blood culture assay for direct detection of Gram-positive organisms and resistance markers in a pediatric hospital. *J Clin Microbiol*. 2014;52(1):283–287.
8. Vareechon C, Mestas J, Polanco CM, Bard JD. A 5-year study of the performance of the Verigene Gram-positive blood culture panel in a pediatric hospital. *Eur J Clin Microbiol Infect Dis*. 2018;37(8):2091–2096.
9. Beckman M, Washam MC, DeBurger B, et al. Reliability of the Verigene system for the identification for Gram-positive bacteria and detection of antimicrobial resistance markers from children with bacteremia. *Diagn Microbiol Infect Dis*. 2019;93(3):191–195.
10. Claeys KC, Heil EL, Pogue JM, et al. The Verigene dilemma: Gram-negative polymicrobial bloodstream infections and clinical decision making. *Diagn Microbiol Infect Dis*. 2018;91(2):144–146.